Growth, Metamorphosis, and Gape-limited Cannibalism and Predation on Tadpoles in Larvae of Salamanders *Hynobius retardatus*

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ABSTRACT—Growth, metamorphosis, and gape-limited cannibalism and predation on tadpoles (*Rana pirica*) in larvae of salamanders (*Hynobius retardatus*) were investigated in laboratory. Larval period and the size at metamorphosis were correlated positively to one another in the salamanders. When embryos were exposed to low temperature, larval period were prolonged and the size at metamorphosis increased in the salamanders while larval period were extended and the size at metamorphosis decreased in the frogs. Salamander larvae reared in group had shorter larval period and smaller size at metamorphosis than those reared individually. Small salamander larvae were more vulnerable to cannibalism and mutilation than large ones. Tadpoles incurred high probability of predation and mutilation by salamander larvae even when the head widths of tadpoles attained the maximum sizes.

INTRODUCTION

Populations and communities of amphibian larvae are regarded as "size-structured" and their intra- and interspecific relationships change with body size rather than age [15–18]. Therefore, growth and metamorphosis (larval period, size, etc.) are essential keys to understand their intra- and interspecific interactions.

Two amphibian species, salamander *Hynobius retardatus* and frog *Rana pirica,* are common and wide-spread in Hokkaido, northern Japan. They spawn at the same sites such as small transient ponds and their larvae co-exist during their larval periods [7, 8]. The salamander larvae eat conspecific larvae and tadpoles of the frog [9], and cannibalism and predation seem to be important intra- and interspecific interactions for them.

Since the success of cannibalism and predation in salamander larvae depends on both of their own gape size and prey's body size [18], larvae of *H. retardatus* and *R. pirica* might regulate growth rates and the timing of metamorphosis to avoid or facilitate cannibalism and predation. On the other hand, growth and metamorphosis of most amphibians are negatively affected by low temperature [1]. The two amphibian species in Hokkaido initiate oviposition in early spring immediately after snow begins to melt [7, 10]. Thus, their eggs or embryos may experience low temperature, and it is desirable to examine the effects of low temperature during embryonic stages on larval growth and metamorphosis in order to investigate intra- and interspecific interactions of these amphibians.

Few studies of larval growth and metamorphosis in ecological context have been conducted for *H. retardatus* and *R. pirica* (but see [5], [6]). The aim of this paper is to obtain basic information about the relationships between growth and cannibalism/predation in the salamander larvae.

Herein, I investigated (1) the relationship between larval period and the size at metamorphosis in *H. retardatus,* (2) the effect of low temperature during embryonic stages on larval growth and metamorphosis in *H. retardatus* and *R. pirica,* (3) the effect of the presence of conspecific larvae on larval growth and metamorphosis in *H. retardatus,* and (4) the effect of gape size of *H. retardatus* larvae on the success of cannibalism and predation on *R. pirica* tadpoles.

MATERIALS AND METHODS

Egg sacs of the salamander (*H. retardatus*) and egg masses of the frog (*R. pirica*) were collected on 2–4 May, 1991 at Teshio Experimental Forest of Hokkaido University in northern Hokkaido. The egg sacs and masses were brought to laboratory at Sapporo campus of Hokkaido University, and experiments were conducted from May to September, 1991.

Three clutches of salamanders were used to examine the relationship between larval period and the size at metamorphosis. The three clutches consisted of 32, 41, and 26 larvae, for each. Larvae were reared with their siblings in polyethylene containers (26 × 18 × 8 cm, 3600 ml water contained) under uncontrolled room condition (19–23°C in spring and 22–26°C in summer, natural day light).

Three experiments were designed for growth studies of the salamander larvae. *Experiment 1 (moderate temperature treatment, individually reared).* After most larvae in a clutch had hatched in a room condition (19–23°C, natural day light), 16 larvae chosen randomly from the clutch were kept separately in glass vials (5-cm diameter and 10-cm height, 180 ml water) under 20°C and 16L8D (16 hr light and 8 hr dark period cycle) condition. *Experiment 2 (low temperature treatment, individually reared).* After introduced into laboratory, a pair of egg sacs (=a full clutch) was kept in a refrigerator (4–7°C, all dark period) for 20 days. Most embryos in the egg sacs were in the tail-bud stage at the beginning of the

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treatment. Then, the egg sacs were brought into the room condition. Some larvae began to hatch within one day. After most larvae had hatched, 16 larvae randomly chosen were separately kept in the glass vials under 20°C and 16L8D condition. *Experiment 3 (low temperature treatment, reared in group).* Descriptions were the same as in Experiment 2 except that 20 larvae were reared together in a polyethylene container (3600 ml water). In the container, some pieces of plastic net were put so that larvae could rest on them and escape from severe antagonistic interactions by other larvae. Individuals were not identified in this experiment. Larvae examined in Experiments 2 and 3 were from the same clutch, and mean total length and head width were not different significantly between the two groups at the beginning of the experiments (Mann-Whitney's U $=142$, $P=0.567$). Artificial feeds for salmon fry were given for larvae in early stages and dried *Tubifex* for developed ones. Foods were given every 1–2 day, and a given amount of food per capita was the same for all experimental designs. Water was replaced every 2 days.

Two experiments were designed for growth studies of the tadpoles. *Experiment 1 (moderate temperature treatment, individually reared).* After most tadpoles had hatched, 16 tadpoles were chosen randomly and kept separately in glass vials (180 ml water). *Experiment 2 (low temperature treatment, individually reared).* After introduced into laboratory, a part of egg mass was kept in a refrigerator (4–7°C, all dark period) for 17 days, and then brought into a room condition (19–23°C, natural day light). The embryos in the egg mass were in the late tail-bud stage at the beginning of the treatment, and some embryos began to hatch one day before the introduction to the room condition. After most tadpoles had hatched, 16 tadpoles were kept separately in the glass vials under 20°C and 16L8D condition. Both in Experiments 1 and 2, kinship among tadpoles was unknown. Artificial feeds for salmon fry were given ad libitum every day. Water was replaced every 1–2 days.

For the experiments of cannibalism and predation on tadpoles in salamander larvae, salamander and frog larvae in various sizes were prepared by controlling water temperature. Experimental procedures were as follows. Larvae were introduced separately in glass vials under 20°C and 16L8D condition, and no foods were provided for two days. After body size (head width and total length) was measured, two individuals (salamander-salamander or salamander-tadpole combination) were introduced into a glass vial (180 ml water). Three types of interactions, predation/cannibalism, mutilation (including killing but not eating a whole body), and non-antagonism, were recorded 24 hours later. Kinship among salamander and frog larvae was unknown.

Definitions of body measures are as follows. Total length, length from the anterior point of head to the posterior point of tail; head width, the maximum width of head without gills. Total length and head width were measured by digital vernier calipers to the nearest 0.1 mm, and wet weight by a micro-electrobalance to the nearest 1 mg. In this paper, 'metamorphosed' refers to the condition that gills became degenerated and skins began to be melanised for salamander larvae and that total length attained the maximum size for tadpoles. Larval period was defined as the period from the day when larvae began to hatch in a clutch to the day when an individual larva metamorphosed.

RESULTS

Growth and metamorphosis of salamanders

Mean time lag between the first and last hatching dates was 4.7 days (N=11, SD=1.10, Range=2–6). In a clutch, hatching began on May 27 and continued until May 31. The majority of larvae hatched on the first and second days; 25 larvae on May 27, 31 on May 28, and 5 on May 29–31. The hatchlings which emerged on earlier days had significantly smaller total length and head width than those hatched on later days (Table 1; Mann-Whitney's U-test, $P<0.003$).

TABLE 1. Total length and head width (mm) of hatchlings in a clutch of salamander *Hynobius retardatus* on different dates. The egg sac was kept in uncontrolled room condition. Hatchlings were removed from water tank immediately after they hatched and then fixed in 10% formalin.

Date	Total length Mean±SD (N)	Head width Mean±SD (N)
May 27	15.8±0.7 (25)	2.6±0.3 (23)
May 28	16.5±0.8 (31)	3.2±0.3 (30)
May 29–31	18.7±1.4 (5)	4.0±0.4 (5)

Head width continued to increase until metamorphosis in some larvae, but other larvae showed abrupt decline of head width just before metamorphosis (Fig. 1). The power function curves showed good fit to the growth trajectories of head width. The power function curve is defined as follows; $Y= aX^b$, where X is period (day) from the first hatch day of a clutch, Y the head width for X, a and b constants. The constants were calculated by the least square method. I also applied the exponential curve ($Y=ae^{bX}$; a and b, constants), which was often used as a growth model of amphibian larvae (e.g., [17], [19]), but it fitted worse than the power function curve.

Larval period and the size at metamorphosis (total length, head width, wet weight) were correlated positively with each other, and all the regression lines were significant (ANOVA, $P<0.001$). However, correlation was rather weak in head width (R=0.41) than in total length (R=0.67) and wet weight (R=0.65). Furthermore, the third ordered regression curves fitted better (ANOVA, $P<0.0001$) and had higher R^2 values than the linear and second ordered regressions in all the body size dimensions. The two-way plot for larval period and the wet weight at metamorphosis was shown as a representative in Figure 2.

The body size at metamorphosis (total length, head width, wet weight) tended to be larger for the low temperature treatment than for the moderate temperature treatment (Table 2, Exp. 1 *vs.* 2), and wet weight showed significant difference between them ($U= 34.0$, $P<0.02$). Larval period was longer significantly for the low temperature treatment than for the moderate temperature treatment (Table 2; $U=12.5$, $P<0.004$).
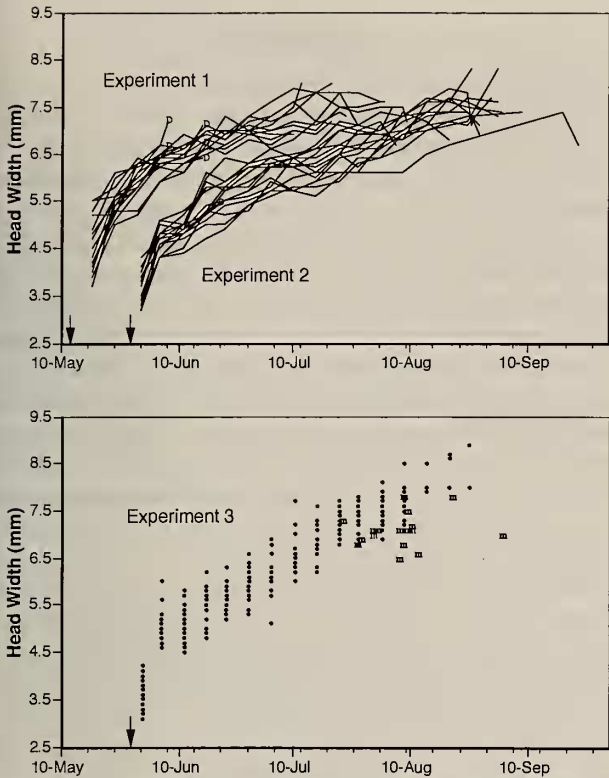
Fig. 1. Head width until metamorphosis for individual larvae of salamanders *Hynobius retardatus* in three experiments. Experiment 1, separately reared and moderate temperature treatment during embryonic stages; Experiment 2, separately reared and low temperature treatment; Experiment 3, reared in group and low temperature treatment. Individuals were not identified in Experiment 3. All larvae were reared under 20°C and 16L8D condition. Larvae within each experiment were from the same clutches, and larvae of an identical clutch were used in Experiments 2 and 3. Arrows indicate the first hatch days. Marks "D" and "m" denote death before metamorphosis and metamorphosis, respectively.
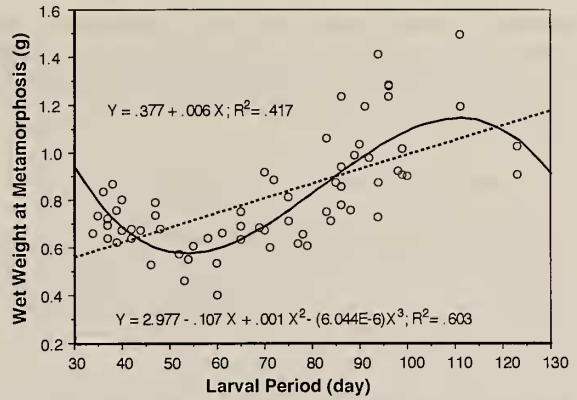

Fig. 2. Two-way plot of larval period and the wet weight at metamorphosis in salamanders *Hynobius retardatus*. Larvae were reared with their siblings under uncontrolled room conditions. Both the regression line and the third-order regression curve were significant ($P < 0.001$).

The larvae reared in group had significantly shorter larval period and smaller wet weight and head width at metamorphosis than those reared individually (Table 2, Exp. 2 *vs.* 3; $U = 25.5$ for period, 22.0 for wet weight, and 73.5 for head width, $P < 0.05$), but total length demonstrated no significant difference between the larvae reared in group and individually ($U = 80.0$, $P < 0.054$). No larvae were eaten by conspecifics during Experiment 3, but one larva died before metamorphosis.

Growth and metamorphosis of tadpoles

Growth trajectories for head width until metamorphosis were fitted well to the power function curves, $Y = aX^b$ (ANOVA, $P = 0.01$). I also applied the exponential curve but they fitted worse than the power function curve.

Mean larval period was significantly longer for the low temperature treatment than for the moderate temperature

TABLE 2. Larval period and the size at metamorphosis in salamanders *Hynobius retardatus* in three experiments. Experiment 1, separately reared and moderate temperature treatment during embryonic stages; Experiment 2, separately reared and low temperature treatment; Experiment 3, reared in group and low temperature treatment

Experiment	N	Period (day)	Total length (mm)	Head width (mm)	Wet weight (g)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	11	73.9 ± 10.0	48.8 ± 2.4	7.4 ± 0.4	0.733 ± 0.124
2	14	96.0 ± 11.1	49.1 ± 3.0	7.4 ± 0.5	0.895 ± 0.161
3	19	71.4 ± 9.3	47.1 ± 2.8	7.1 ± 0.3	0.623 ± 0.101

TABLE 3. Larval period and the size at metamorphosis in frogs *Rana pirica* in two experiments. Experiment 1, separately reared and moderate temperature treatment during embryonic stages; Experiment 2, separately reared and low temperature treatment

Experiment	N	Period (day)	Body length (mm)	Head width (mm)
		Mean ± SD	Mean ± SD	Mean ± SD
1	15	37.3 ± 3.0	42.5 ± 2.3	8.6 ± 0.7
2	10	40.0 ± 2.1	34.7 ± 1.4	7.9 ± 0.3

treatment ($U=22.5$, $P=0.0036$), while mean size at meta-morphosis was significantly smaller for the former than for the latter ($U=0.0$ for total length and 26.0 for head width, $P<0.01$) (Table 3).

Gape-limited cannibalism and predation

No cannibalism occurred in salamander larvae when both larvae had similar head width, but mutilation occurred even when opponents were much larger (Fig. 3). Small larvae were more vulnerable to cannibalism than large ones.

When salamander larvae were small, tadpoles were not eaten by them even if head widths of tadpoles were smaller than those of salamanders (Fig. 4). A number of tadpoles were eaten or mutilated by the salamander larvae whose head widths were larger than approximately 4.5 mm, even if head widths of tadpoles were larger than those of salamanders. No tadpoles could escape from predation nor mutilation by the salamanders larger than approximately 7.5 mm.
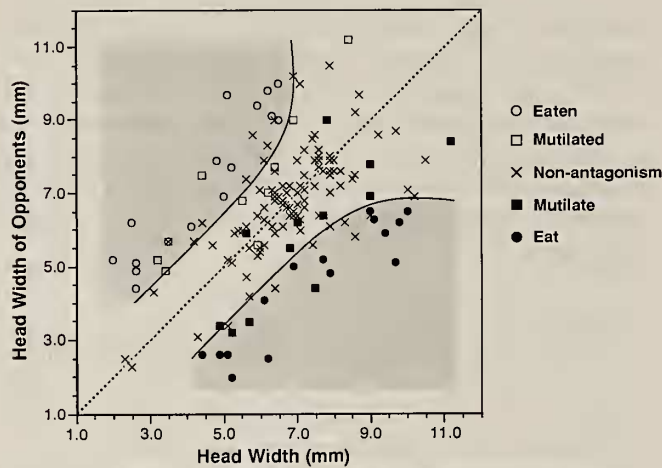

FIG. 3.   Head width and cannibalism in larvae of salamanders *Hynobius retardatus*. Shaded areas indicate the regions of high probability of cannibalism (curves were drawn by hand). Broken line is an isoline.
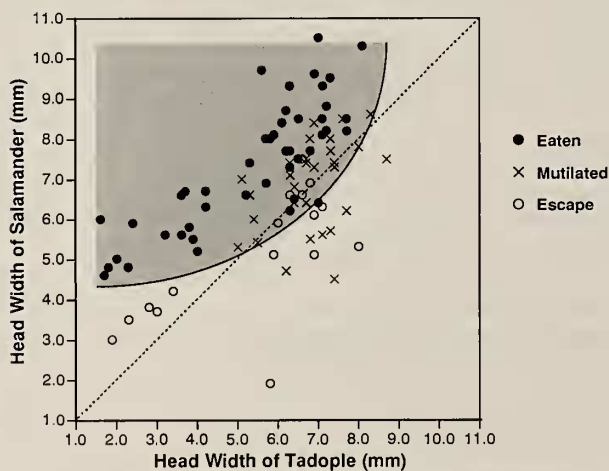

FIG. 4.   Head width and predation in larvae of frogs *Rana pirica* and salamanders *Hynobius retardatus*. Shaded area indicates the region of high probability of predation (a curve was drawn by hand). Broken line is an isoline.

DISCUSSION

Relationship between larval period and size

Larval period and the size at metamorphosis were corre-lated positively with each other in *H. retardatus* (Fig. 2). A similar relationship has been reported in many amphibian species [1, 3, 5]. The third ordered regression curve was more explainable for the scattering of the data than the linear regression line (Fig. 2). I interpreted the formation of the third ordered function curves as follows. First, the larvae with higher growth rates begin to metamorphose. Subsequent-ly, those with lower growth rates begin to metamorphose until the minimum point of the curve. The larvae that could not metamorphose by the minimum point decide to prolong their larval periods and grow more. The minimum point might be the compromise point between negative effects of long larval period, such as risk of pond desiccation and higher probability of predation [4, 15, 17, 18], and positive effects of large body size, such as high resistance against starvation and desiccation or high fertility in adults [13]. In contrast, the meaning of the maximum point of the curve is vague. In natural conditions, some larvae of *H. retardatus* over-winter in natural pond [9]. Thus, the larvae with extremely low growth rates will not metamorphose in the year of birth.

Effect of conspecifics on metamorphosis and cannibalism

It has been reported that the presence of other conspe-cific individuals caused long larval period and low growth rate owing to interference competition in salamander *Ambystoma opacum* [12] and anurans [2, 11, 14]. In contrast, *H. retarda-tus* demonstrated short larval period when they were reared in group (Table 2). The shortened larval period might be a tactics to avoid cannibalism as Wilbur [18] insisted; small larvae are more vulnerable to cannibalism than larger ones (Fig. 3), and short larval period seems to be a solution for the avoidance of cannibalism. In addition, it could be also regarded as the avoidance of cannibalism among siblings that small larvae hatched earlier than large ones (Table 1).

Low temperature effects on metamorphosis

It has been known that metamorphosis postponed when larvae were reared in low temperature in many species of amphibians [1] including *Hynobius* spp. and *R. pirica* [3, 5, 6]. In the present study, it was found that low temperature exposure during embryonic stages also prolonged larval periods in the amphibians even when larvae were reared in moderate temperature (Tables 2 and 3). The prolongation of larval period was accompanied by large body size at metamorphosis in *H. retardatus* (Table 2), while it occurred with small body size in *R. pirica* (Table 3). Therefore, low temperature in early spring seems to be more malignant for *R. pirica* than *H. retardatus*, since the latter could deny the negative effects of long larval period by the positive effects of large metamorph size while the former could not.

Predation on tadpoles by salamanders

Even large tadpoles of *R. pirica* have high probability of predation and mutilation by the salamander larvae unless salamander larvae are in early stages (Fig. 4). Tadpoles can lessen the risk of predation or mutilation by hatching earlier and/or leaving ponds earlier than the salamanders. Certainly, many *R. pirica* oviposit earlier than *H. retardatus* in Tokachi region [10], although oviposition in early spring may be in danger of low temperature exposure during embryonic stages, which has unfavorable effects (long larval period and small metamorph size) at metamorphosis for them (Table 3). Period for hatching is shorter in *R. pirica* than *H. retardatus* at 10 and 20°C [5], and growth rate until metamorphosis of *R. pirica* was much higher than that of *H. retardatus* (Fig. 1 and Table 3). Therefore, most of *R. pirica* appear to leave ponds earlier than *H. retardatus*.

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